USAMRIID

Evaluating the influences of glycosylation on the antigenicity and immunogenicity of Ebola virus glycoprotein

W. Dowling, E. Thompson, J. Paragas, W. Capps, C. Badger, and C. Schmaljohn

U.S. Army Medical Research Institute of Infectious Diseases

R. Hogan

Southern Research Institute

Report Documentation Page

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
15 NOV 2004	**N/A**	**-**

4. TITLE AND SUBTITLE	5a. CONTRACT NUMBER
Evaluating the influences of glycosylation on the antigenicity and immunogenicity of Ebola virus glycoprotein	5b. GRANT NUMBER
	5c. PROGRAM ELEMENT NUMBER
6. AUTHOR(S)	5d. PROJECT NUMBER
	5e. TASK NUMBER
	5f. WORK UNIT NUMBER
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) **U.S. Army Medical Research Institute of Infectious Diseases**	8. PERFORMING ORGANIZATION REPORT NUMBER
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)	10. SPONSOR/MONITOR'S ACRONYM(S)
	11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION/AVAILABILITY STATEMENT
Approved for public release, distribution unlimited

13. SUPPLEMENTARY NOTES
See also ADM001849, 2004 Scientific Conference on Chemical and Biological Defense Research. Held in Hunt Valley, Maryland on 15-17 November 2004 . , The original document contains color images.

14. ABSTRACT

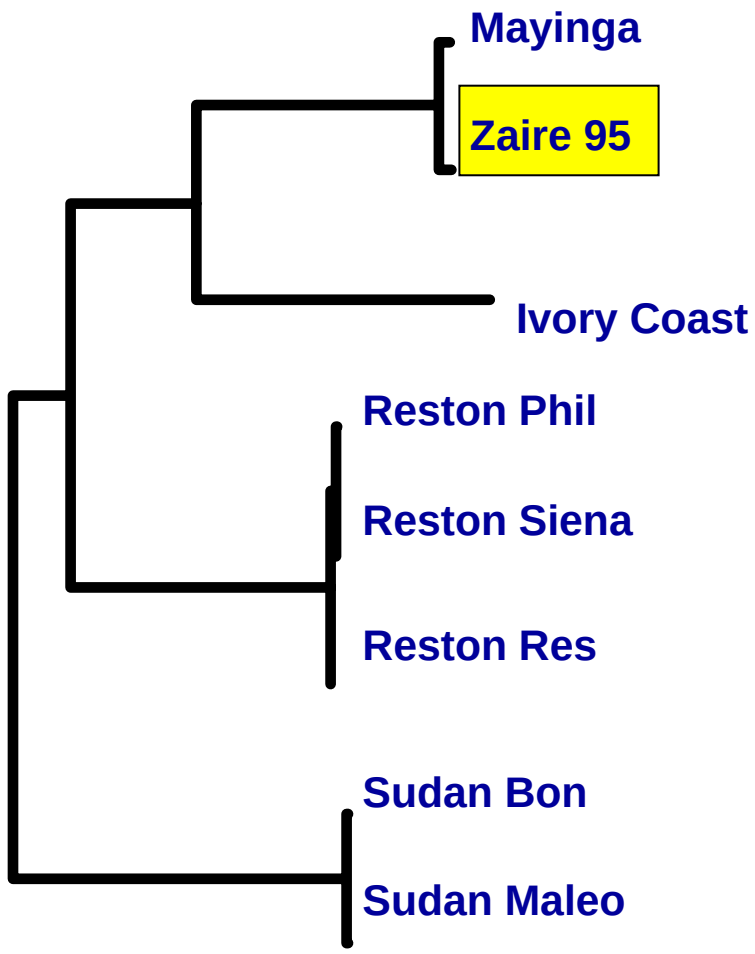
15. SUBJECT TERMS

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			
unclassified	**unclassified**	**unclassified**	**UU**	**31**	

Ebola Virus

- Causative agent of severe hemorrhagic fever
- Identified in Zaire in 1976
- Sporadic outbreaks in Africa
- High mortality rate - 50-88%
- Animal reservoir unknown
- Lack of preventative vaccine or effective antiviral treatments
- Potential bioterrorism agent

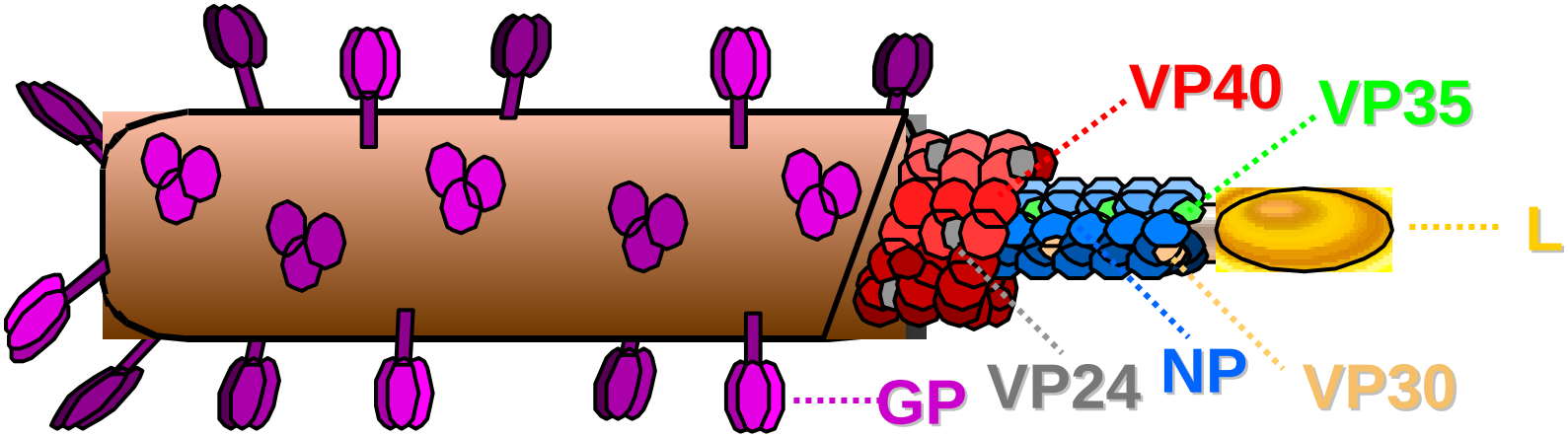
Ebola Virus Strains



Phylogenetic Relationships of GP gene sequence

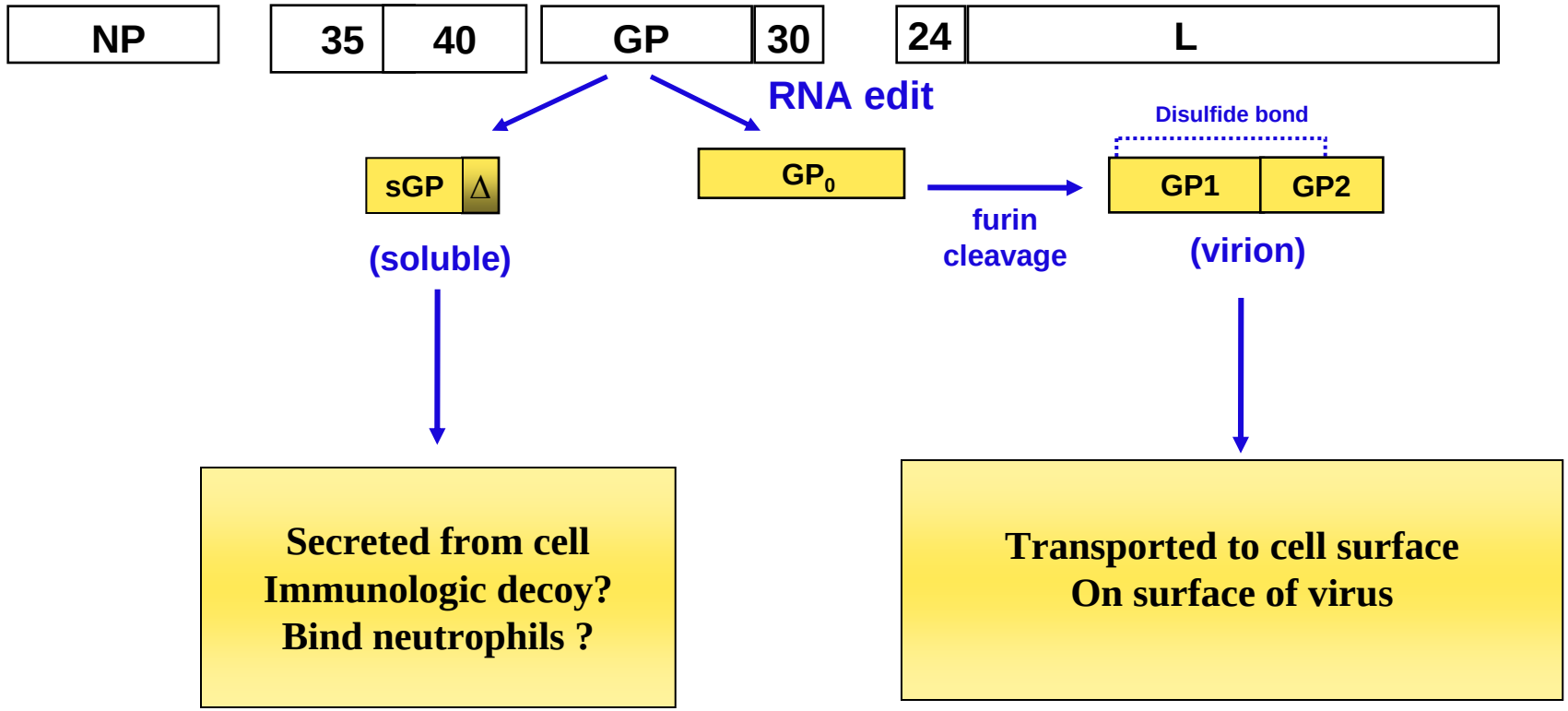
Strain	Origin	#Cases (%Mortality)
Mayinga	Zaire 1976	318 (88%)
Zaire 95	Zaire 1995	315 (77%)
	Gabon 94-96	~100 (50%)
	Gabon/Congo 2002	92 (75%)
	Congo 2003	143 (89%)
Ivory Coast	Ivory Coast 1994	1 (0%)
Reston Phil		
Reston Siena	Philippines 1989	
Reston Res		
Sudan Bon	Sudan 1976	284 (53%)
Sudan Maleo	Sudan 1979	34 (65%)
	Uganda 2000-01	~400 (62%)

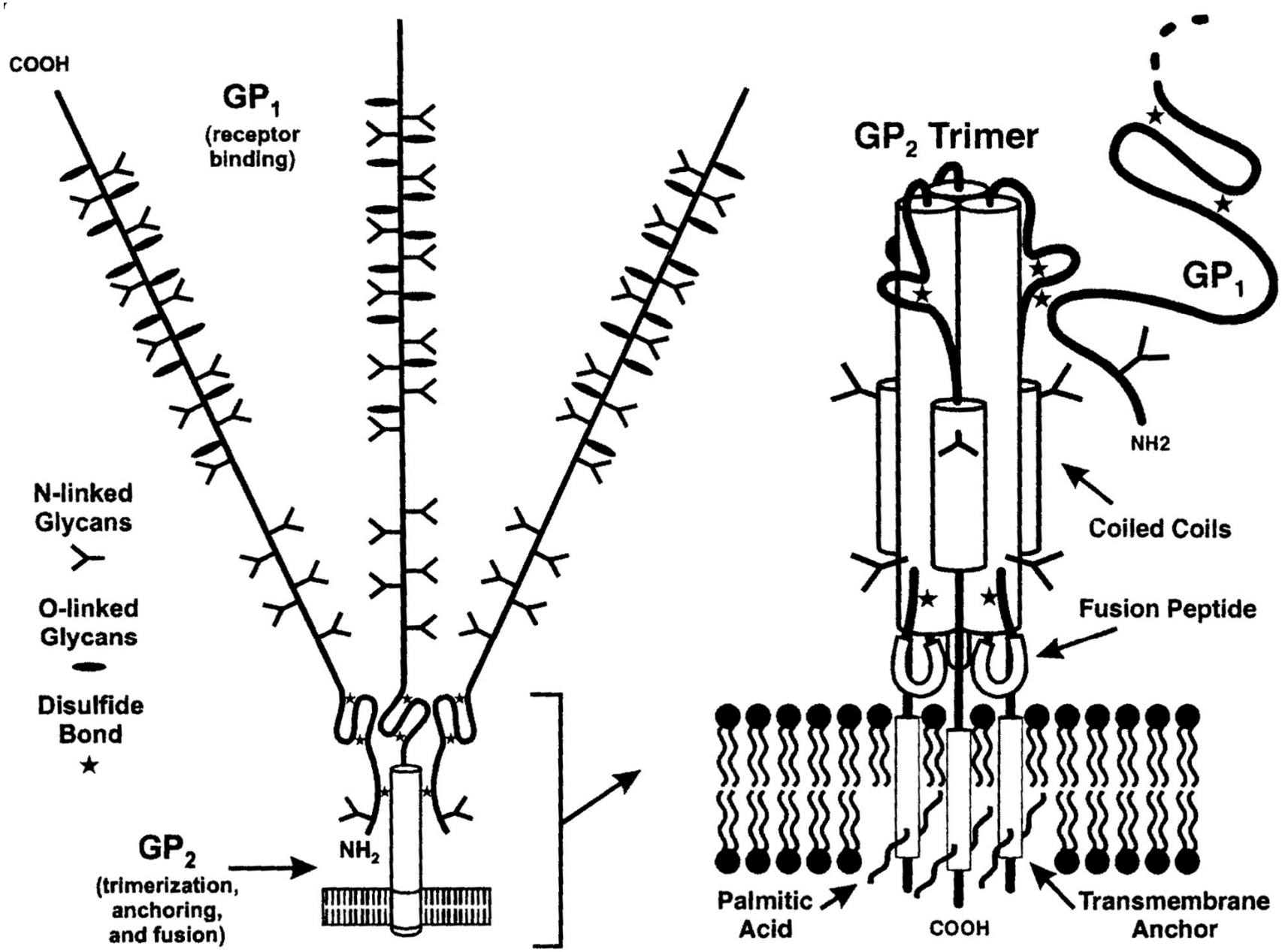
Ebola virus Structure



GP	Transmembrane Glycoprotein
VP40	Matrix Protein
VP24	Matrix Protein (Minor)
NP	Nucleoprotein
VP35	Phosphoprotein (Transcription Factor)
VP30	Ribonucleoprotein Associated (Minor)
L	RNA-Dependent RNA Polymerase

Synthesis of Ebola virus glycoproteins





From Fields Virology 3rd ed p. 1287

N-linked glycosylation

- Co-translational modification
- Eukaryotic cells in the endoplasmic reticulum
- Occurs at a triplet amino acid sequence (SEQUON):

Asn-X-Ser/Thr

- Not all sequons are glycosylated
- Influences protein folding and transport
- Changes in viral glycoproteins can lead to altered immunogenicity (HIV, CAEV, influenza, rabies) and virulence (influenza, NDV, MHV, PERV)

Ebola GP: N-linked Glycosylation sites

- Zaire GP - 17 potential sites
- Other strains:
  - Reston - 15
  - Sudan - 12
  - Ivory Coast - 12
- Conserved sites - 5 sites in GP1, 2 in GP2. Those in GP2 are the only sites identical at all 4 amino acid positions.

N-linked mutation set 1

N-linked glycosylation sites around *known* protective epitopes

- **Set of 5 monoclonal antibodies**
- **Protect mice against EBOV infection**
- **"Protective" epitopes mapped for 3 of the antibodies**
- **Groups 1 and 2 have overlapping epitope in GP1**
- **N-linked glycosylation sites FLANK either side of this epitope**

QUESTION:
Do glycans at either or both of these sites *shield* or *enhance* this epitope?

N-linked mutation set 2

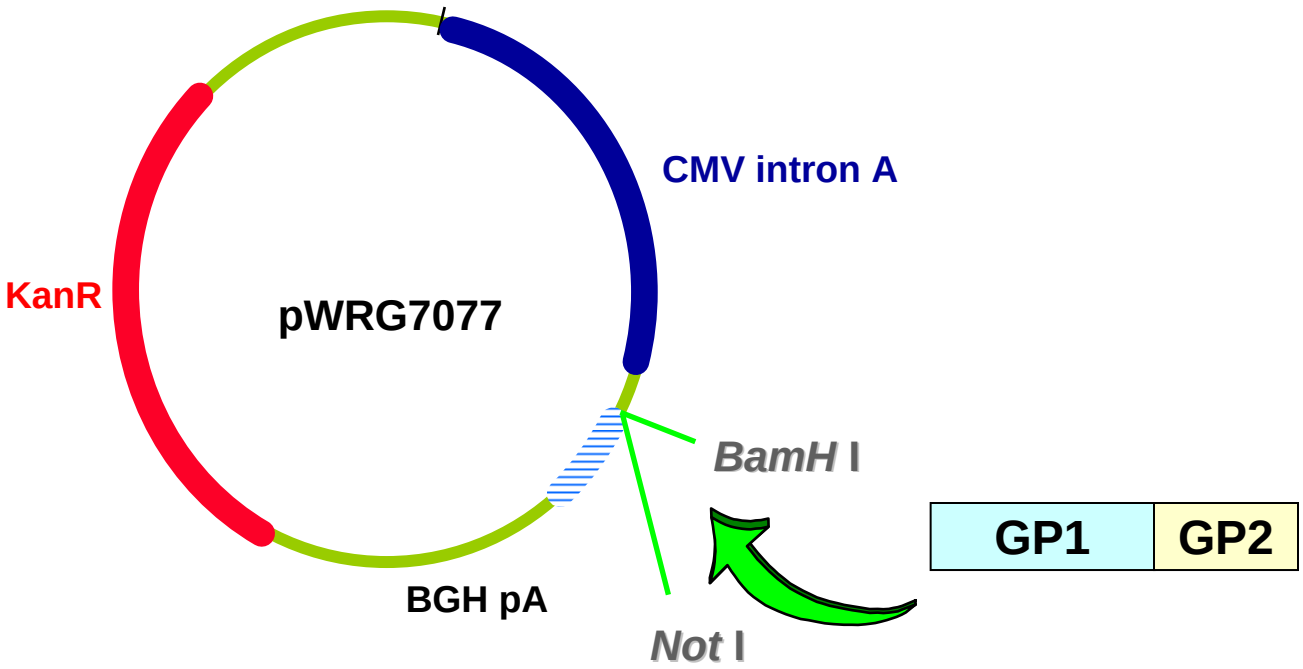
N-linked glycosylation sites in *known* immunogenic regions

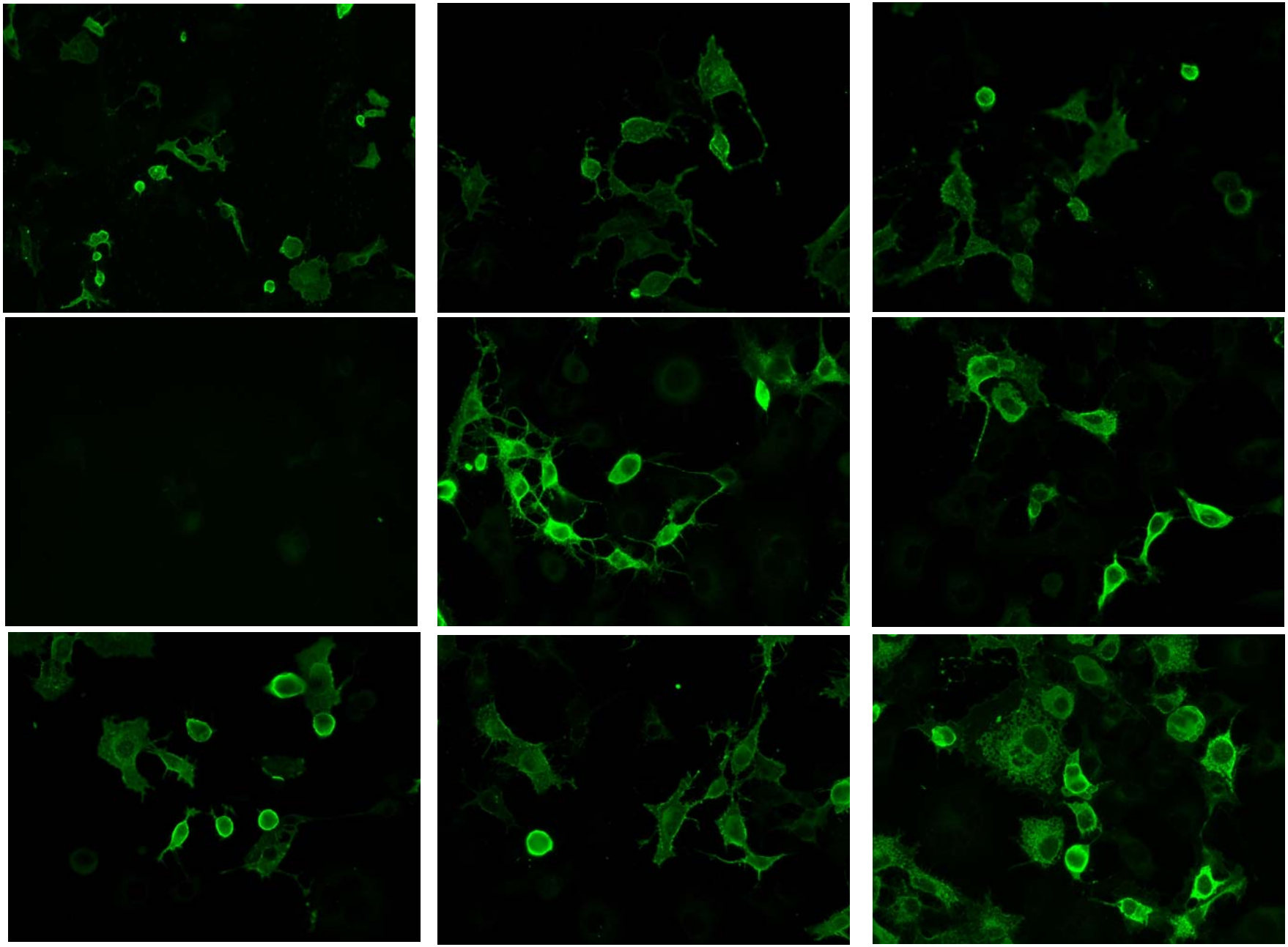
- **GP2 shown to be protective for EBOV and MBGV**
- **Only two sites for N-linked glycosylation in GP2**
- **Highly conserved among different strains/subtypes of EBOV**

QUESTION:
Do glycans in GP2 affect the immunogenicity of this region?

MGVTGILQLPRDRFKRTSFFLWVIILFQRTFSIPLGVIHNSTLQVSDV
DKLVCRDKLSSTNQLRSVGLNLEGNGVATDVPSATKRWGFRSGV
PPKVVNYEAGEWAENCYNLEIKKPDGSECLPAAPDGIRGFPRCRY
VHKVSGTGPCAGDFAFHKEGAFFLYDRLASTVIYRGTTFAEGVVAF
LILPQAKKDFFSSHPLREPVNATEDPSSGYYSTTIRYQATGFGTNET
EYLFEVDNLTYVQLESRFTPQFLLQLNETIYTSGKRSNTTGKLIWKV
NPEIDTTIGEWAFWETKKNLTRKIRSEELSFSAVSNRAKNISGQSPA
RTSSDPGTNTTTEDHKIMASENSSAMVQVHSQGREAAVSHLTTLG
TISTSPQPPTTKPGPDNSTHNTPVYKLDISEATQVEQHHRRTDNDS
TASDTPPATTAAGPLKAENTNTSKGTDLLDPATTTSPQNHSETAGN
NNTHHQDTGEESASSGKLGLITNTIAGVAGLITGGRRARREAIVNA
QPKCNPNLHYWTTQDEGAAIGLAWIPYFGPAAEGIYTEGLMHNQD
GLFCGLRQLANETTQALQLFLRATTELRTFSILNRKAIDFLLQRWGG
TCHILGPDCCIEPHDWTKNITDKIDQIIHDFVDKTLPDQGDNDNWWT
GWRQWIPAGIGVTGVIIAVIALFCICKFVF.

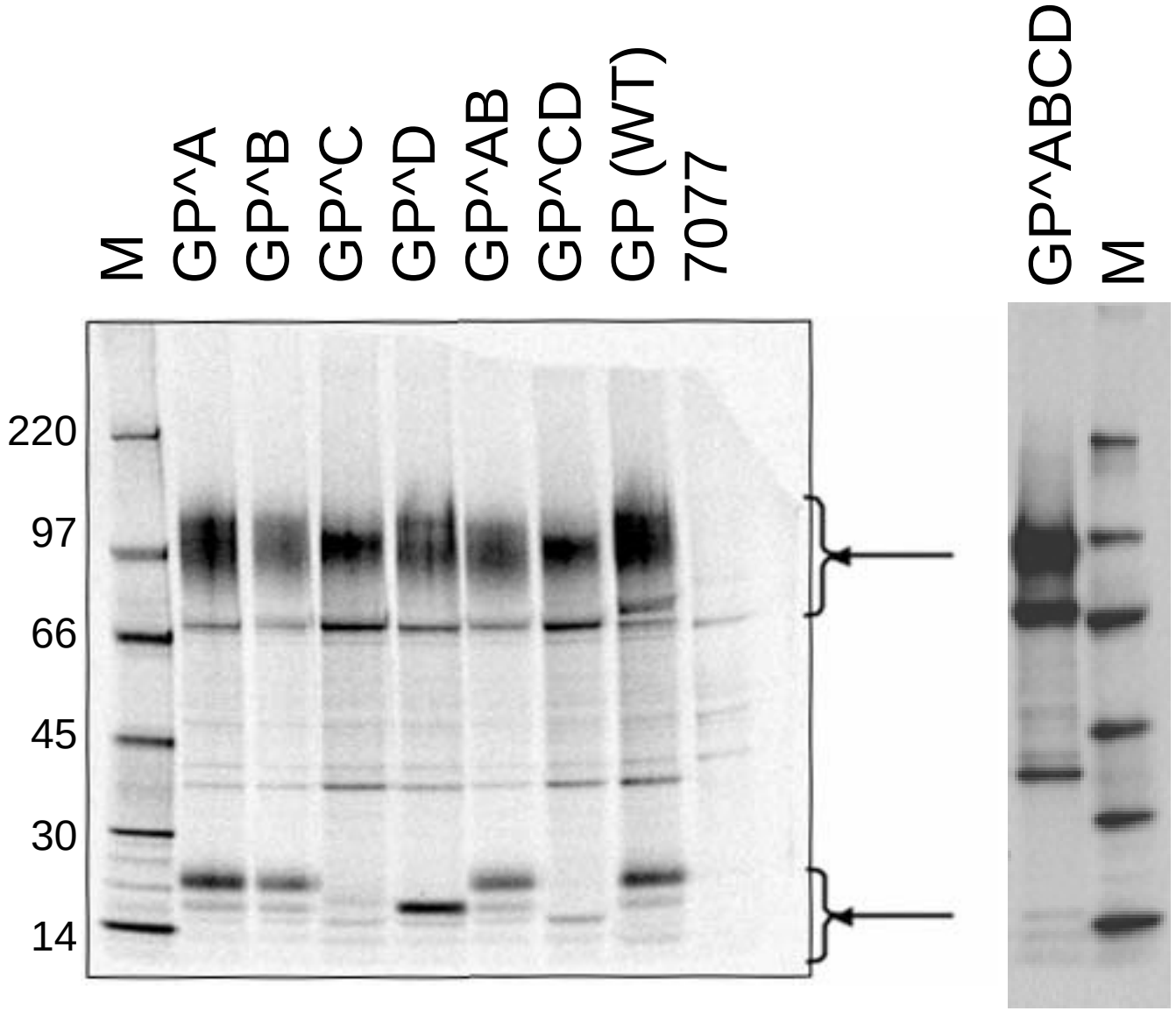
DNA vaccines for EBOV





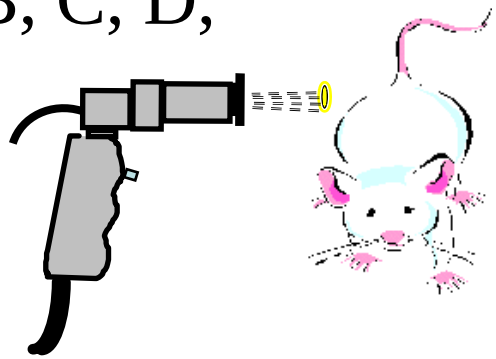
IFA-Fixed Cells

In vitro expression of EBO-Z GP N-linked glycosylation mutants



DNA Vaccination

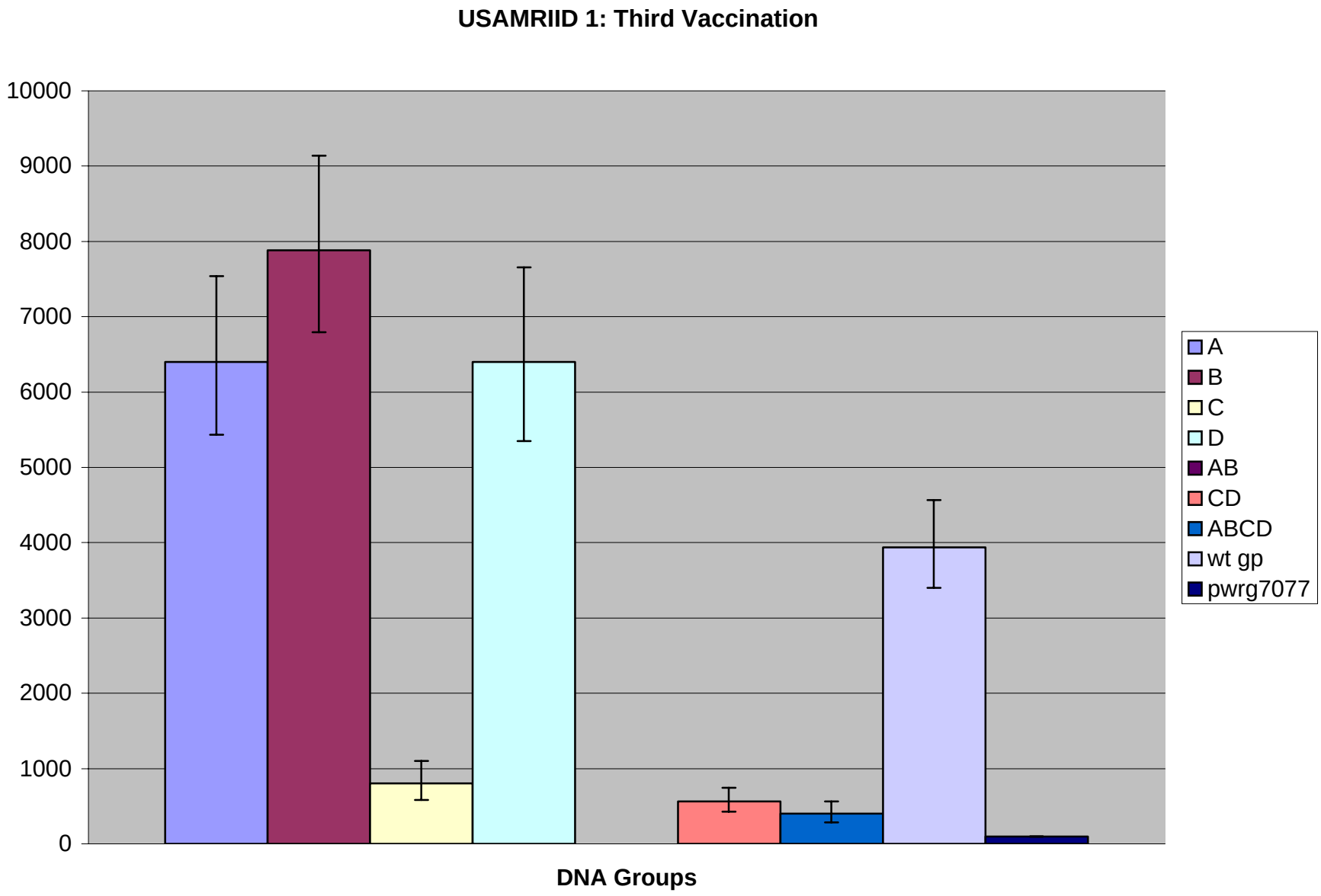
- 9 plasmids: GP wt, pWRG7077, A, B, C, D, AB, CD, ABCD
- 4-6 wk balb/c female mice
- 3 gene-gun vaccinations, 4 wk apart



- Challenge - with mouse-adapted Ebola virus
- ELISA- test sera using irradiated ZEBOV and pools of overlapping peptides as antigens
- ELISPOTs - stimulate with pools of overlapping peptides

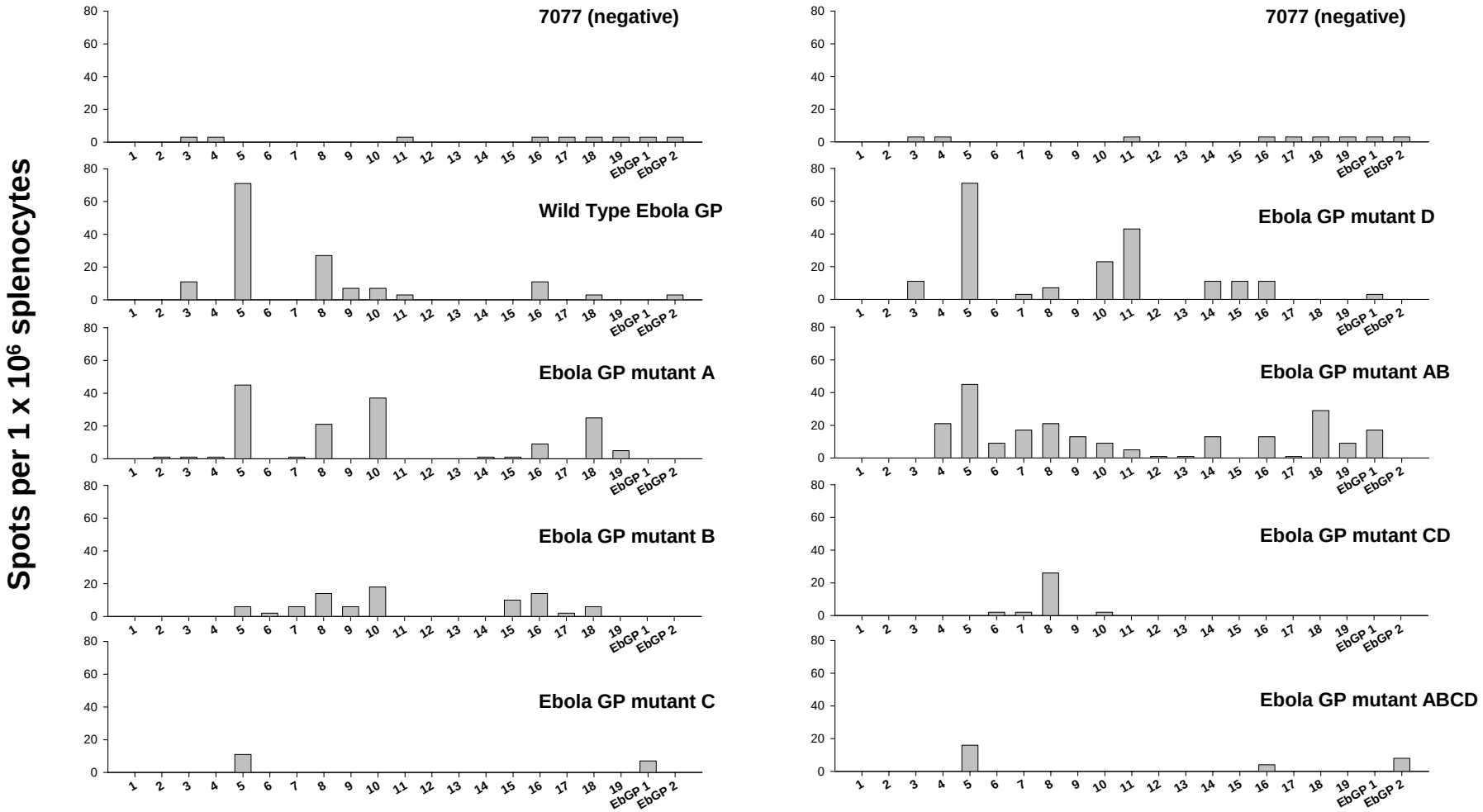
Survival Data

pWRG7077	0/7 (0%)	0/10 (0%)
GP (wt)	7/7 (100%)	10/10 (100%)
GPmutA	7/7 (100%)	10/10 (100%)
GPmutB	7/7 (100%)	9/9 (100%)
GPmutC	3/6 (50%)	6/10 (60%)
GPmutD	7/7 (100%)	9/9 (100%)
GPmutAB	ND	10/10 (100%)
GPmutCD	7/7 (100%)	7/10 (70%)
GPmutABCD	4/6 (67%)	8/10 (80%)


USAMRIID 1: Third Vaccination
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
A
B
C
D
AB
CD
ABCD
wt gp
pwrg7077
DNA Groups

Virus-specific T-cell responses in mice vaccinated with DNA vectors expressing the Ebola GP

Deletion of N-linked glycosylation sites alters T cell specificities and frequencies.



Summary

- All N-linked mutants express when transfected into mammalian cells, as shown by IFA and RIPA. Those containing the “C” mutation at position 586 (C, CD, ABCD) demonstrate a normal level of GP1 but a reduced amount of GP2 by RIP assay.
- Vaccination with wild-type GP or with mutants A, B, D and AB protected mice from challenge with mouse-adapted Ebola Zaire. Mutant C and ABCD afforded partial protection in both sets of experiments while CD was completely protective in the first experiment but only partially protective in the second experiment.
- ELISPOT results showed a decrease in breadth and intensity of T-cell responses from mice vaccinated with mutants C, CD and ABCD. Mutants A, B,D and AB had similar responses to wild type
- ELISA titers were decreased in mutants C, CD and ABCD compared to wild type and elevated slightly in mutants A, B, and D.

Future Directions

- Assess GP2 expression of C-containing mutants by IFA, RIPA and Western Blot
- Peptide ELISA
- Repeat of original 9 groups to test conflicting results (in progress)
- Other mutants: O-linked glycosylation mutants, new N-linked mutants

Acknowledgements

USAMRIID

Dr. Connie Schmaljohn
Elizabeth Thompson
Catherine Badger
SPC Warren Capps
SPC Elena Angoco
Dr. Jason Paragas

SRI

Dr. Robert J. Hogan

FDA

Dr. Jenny Riemneschneider

Lab Animal Usage

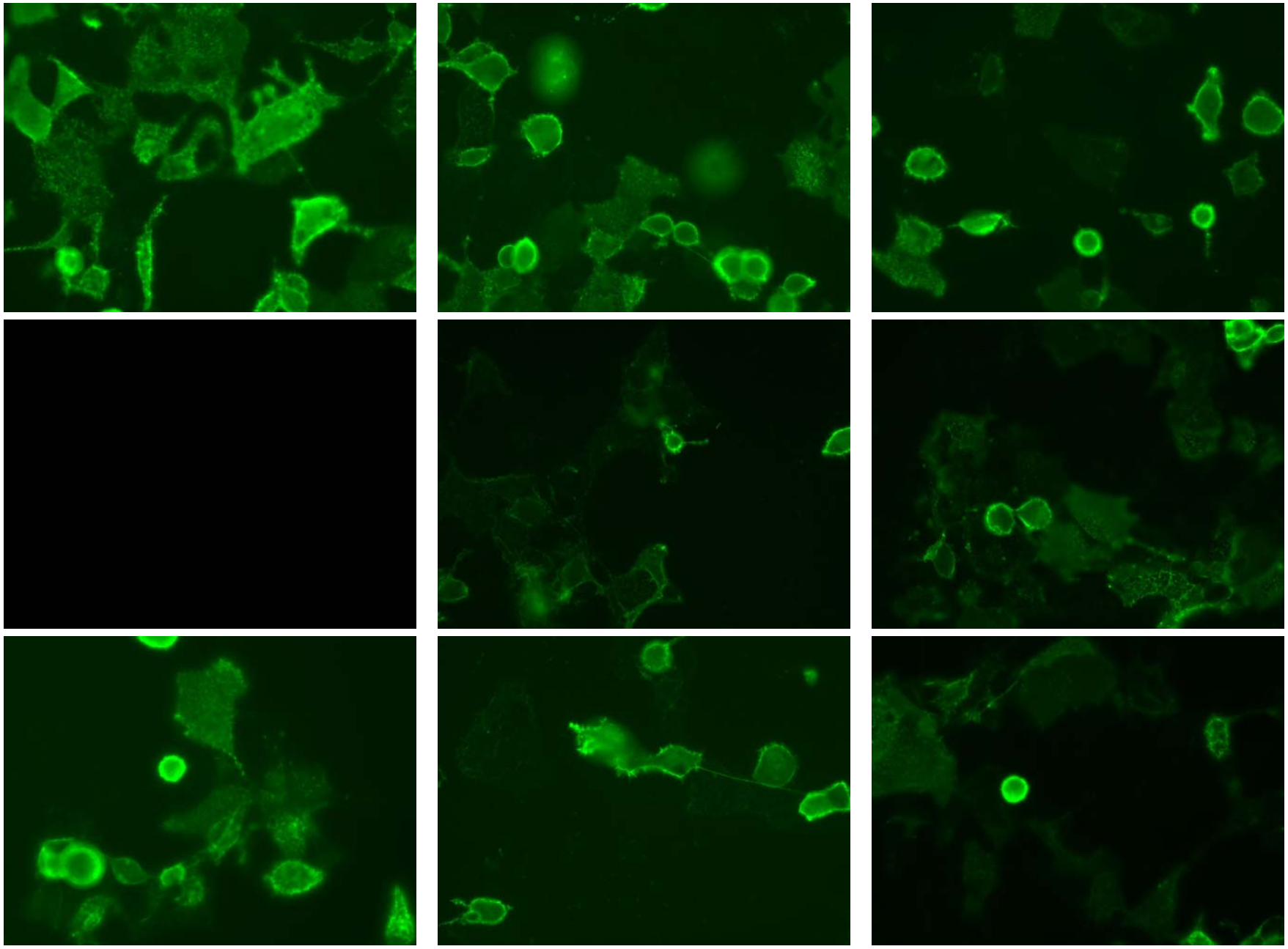
Research was conducted in compliance with the Animal Welfare Act and other Federal statutes and regulations relating to animals and experiments involving animals and adheres to principles stated in the Guide for the Care and Use of Laboratory Animals, National Research Council, 1996. The facility where this research was conducted is fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International.

Disclaimer

Opinions, interpretations, conclusions, and recommendations are those of the author and are not necessarily endorsed by the U.S. Army.

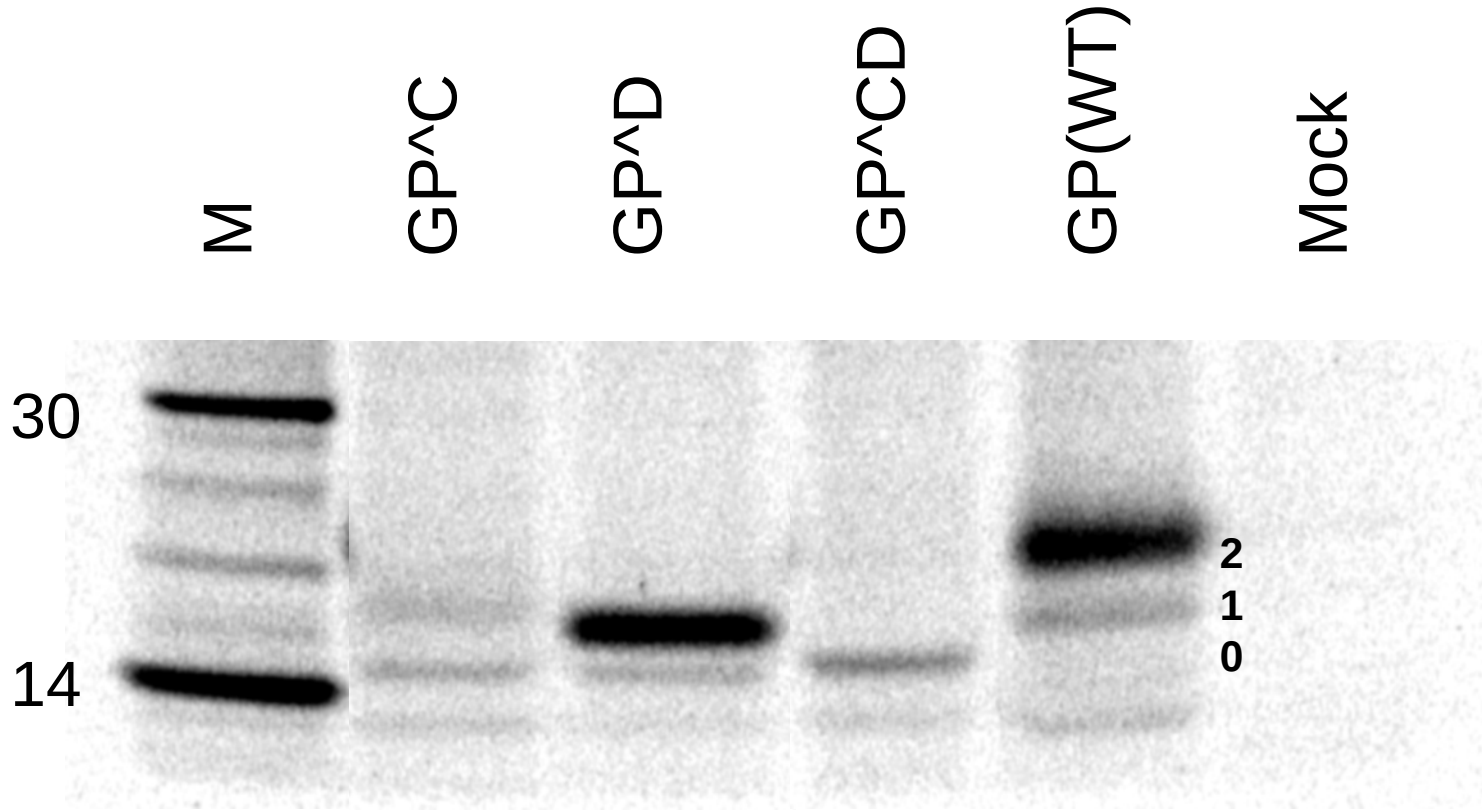
Funding Acknowledgement

The research described herein was sponsored by the Medical Biological Defense Research Program, Project # 02-4-7J-100, Peer Reviewed Medical Research Program and National Biodefense Analysis and Countermeasures Center Project # 04-0-DH-008.



IFA Live Cells

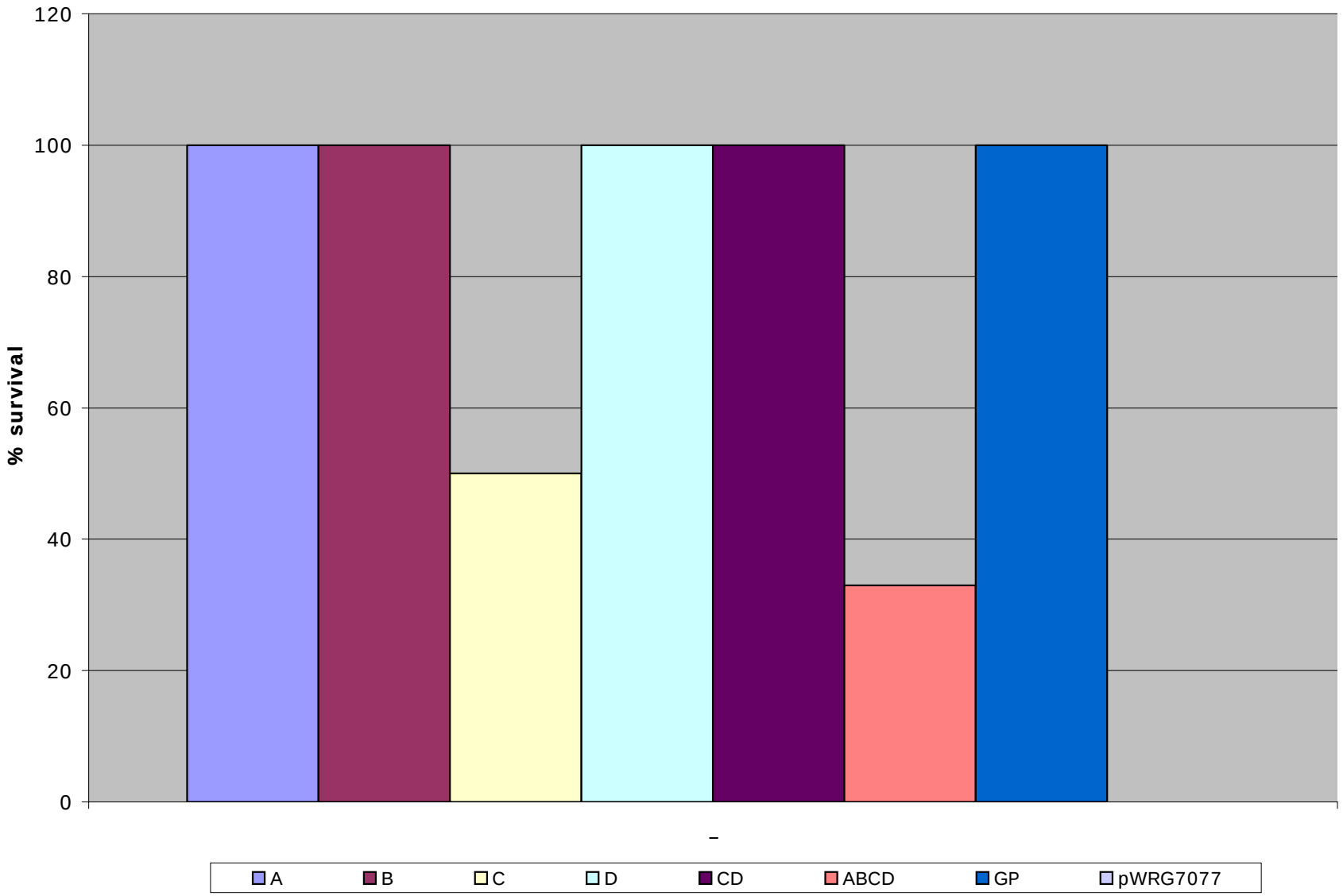
In vitro expression of ZEBO GP N-linked glycosylation mutants

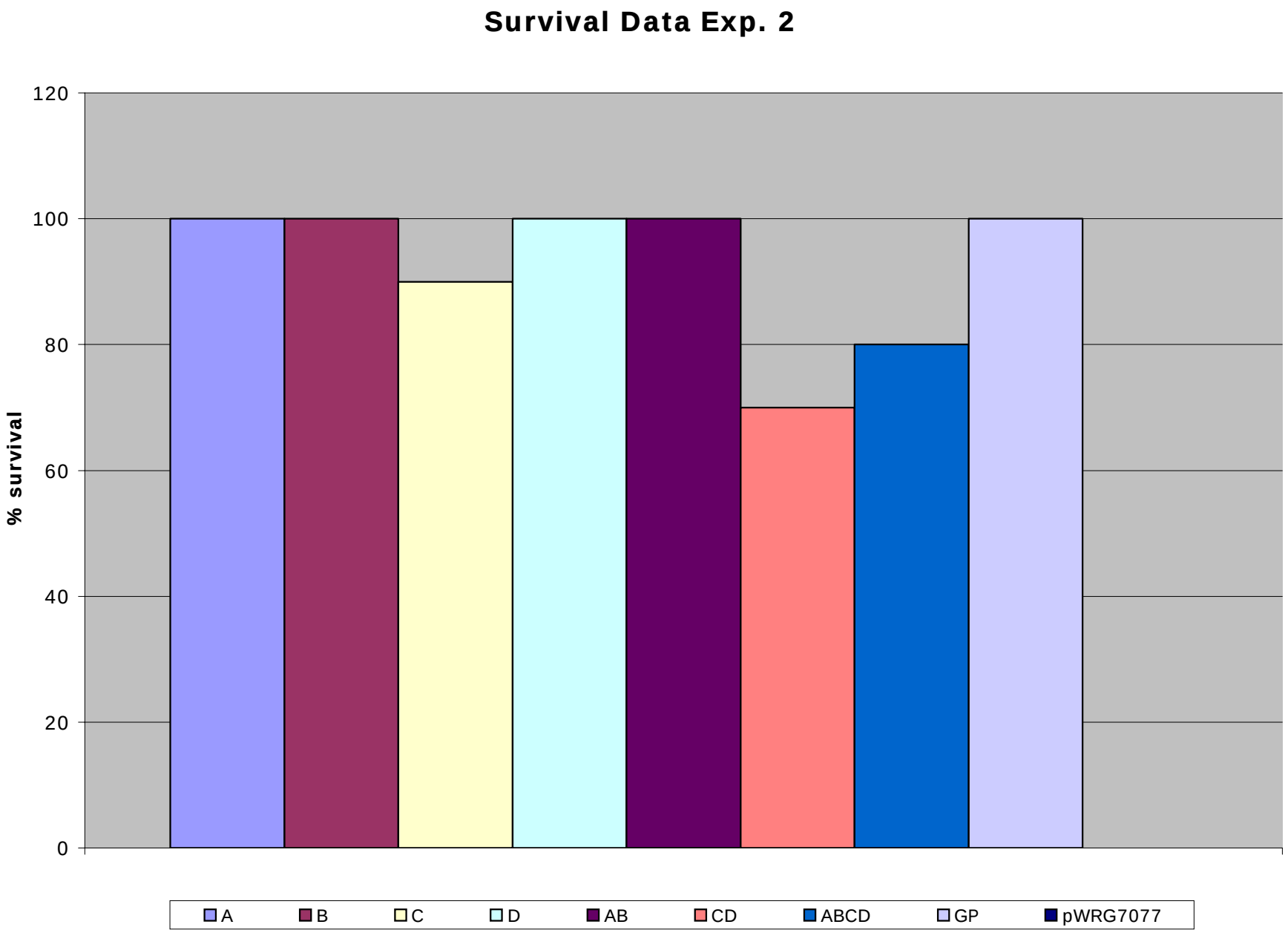


EAIVNAQPKCNPNLHYWTTQDEGAAIGLAWIPYFGPAAEGIY
TEGLMHNQDGLFCGLRQLA**NET**TQALQLFLRATTELRTFSIL
NRKAIDFLLQRWGGTCHILGPDCCIEPHDWTK**NIT**DKIDQIIH
DFVDKTLPDQGDNDNWWTGWRQ<u>WIPAGIGVTGVIIAVIALF
CIC</u>*KFVF.*

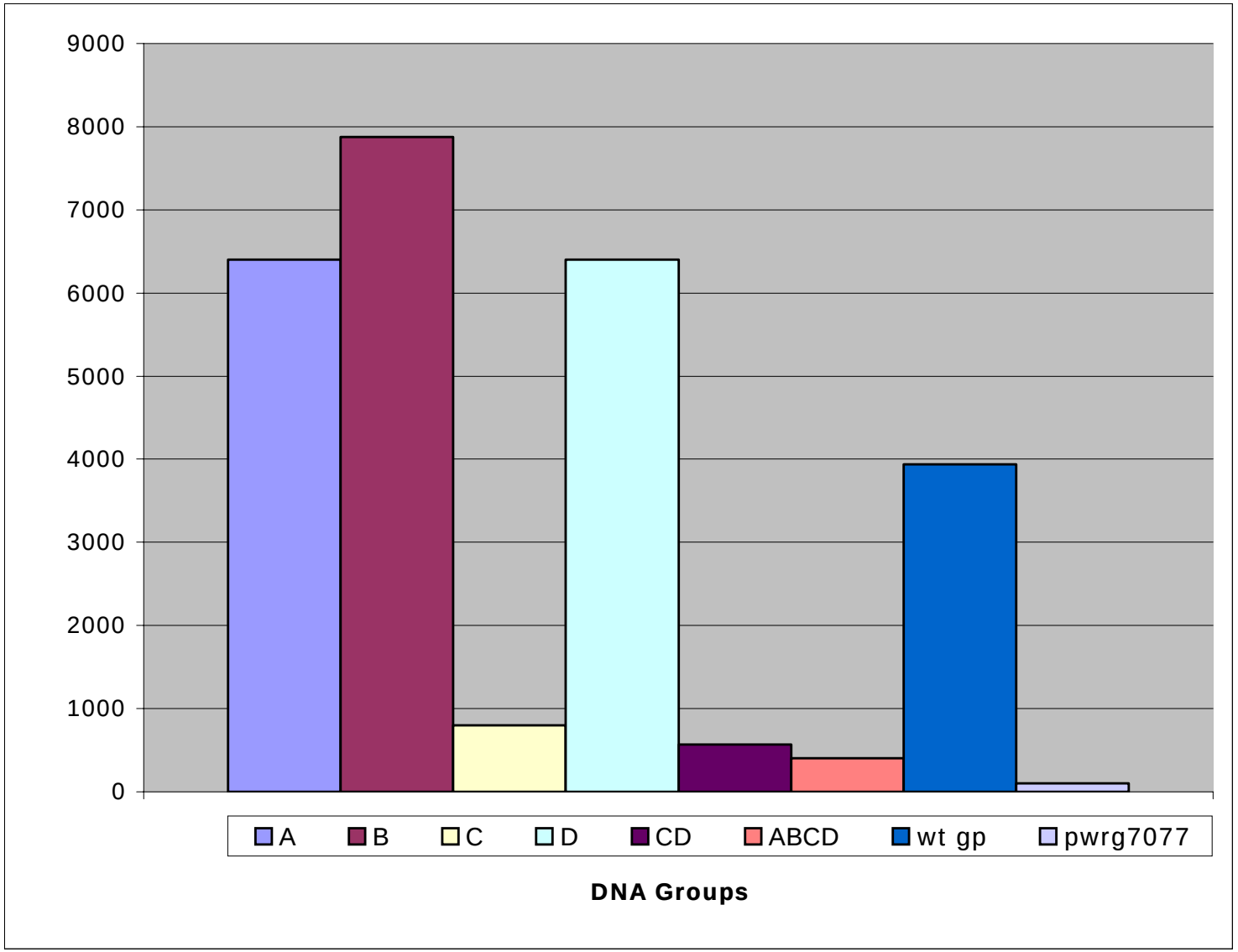
Ebola Virus Vaccines

- GP has been tested as a vaccine candidate in the DNA, VEE replicon, VLP, recombinant VSV and adenovirus vector systems.
- GP protects mice from challenge in all of these systems.
- Both the humoral and cellular immune responses play important roles in protection.

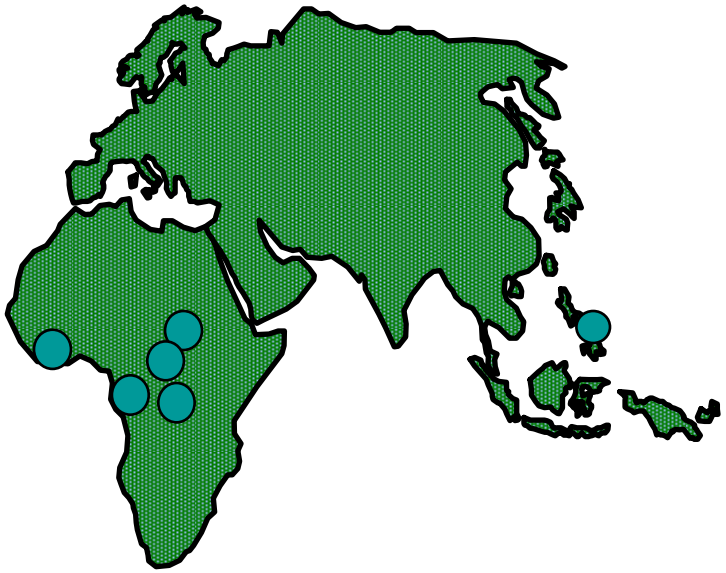

Survival Data Exp. 1
% survival
120
100
80
60
40
20
0
A
B
C
D
CD
ABCD
GP
pWRG7077


Survival Data Exp. 2
% survival
120
100
80
60
40
20
0
A
B
C
D
AB
CD
ABCD
GP
pWRG7077

ELISA results



Ebola Virus



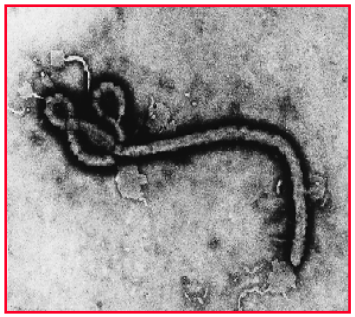
Unknown reservoir

? ?

Monkey

? ?

Human



Filoviridae
enveloped, filamentous ssRNA, (-) >19 kilobases

Fever, Malaise, Diarrhea, Hemorrhage

Ebola Virus Glycoprotein (GP)

- 676 amino acids , approx. 140 kDa
- Post-translation cleavage by furin at AA 501, forms GP1 and GP2 domains
- GP2 has a transmembrane domain and a small intracellular domain
- GP1 remains linked to GP2 by disulfide bonding
- Glycosylation accounts for about half of the molecular weight